International Journal of Agricultural Science and Research (IJASR)
ISSN (P): 2250-0057; ISSN (E): 2321-0087
Vol. 8, Issue 4, Aug 2018, 121-130

DNA BARCODING FOR DIFFERENTIATING THE 11 VARIETIES OF *MUSA* SPECIES

SAIFULDEEN AHMED HASAN & SHAIK MAHAMMAD KHASIM

Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

ABSTRACT

The present investigation was aimed to evaluate the ITS 2 as a mini-barcode for analyzing the phylogenetic relationship among the 11 varieties of Musa species. DNA barcoding was performed by ITS sequencing employing the ITS 2 primer. All the resultant sequences were submitted to NCBI database to attain GenBank accession numbers. MEGA 6.06 software was employed for ITS sequences to determine the phylogenetic relationship among the 11 varieties of Musaspp by Neighbor-Joining method. The primer ITS 2 generated a mini-barcode for all the 11 varieties of Musa spp. and the GenBank accession numbers were obtained for all the sequences from the NCBI database. The 11 varieties of Musa spp. clustered into 3 groups in the phylogenetic tree.

KEYWORDS*: *Musa Spp, Banana, DNA Barcoding, ITS Sequencing & Phylogenetic Analysis

Received: May 07, 2018; **Accepted:** May 28, 2018; **Published:** Aug 03, 2018; **Paper Id.:** IJASRAUG201816

Original Article

INTRODUCTION

Banana belongs to the genus *Musa* which is regarded as the most important tropical fruit that accounts for the global production of 40 million tonnes (FAO, 2003). Banana is also the second most important vital crop in India next to mango (Swain *et al.*, 2016). The high nutritive value and low cost increase the demand for banana compared to other fruits. Banana is a rich source of vitamins especially vitamin B and also contains an appreciable amount of potassium, phosphorous, magnesium and calcium and hence it regarded as a nutritive fruit. The banana is easily digestible since it is free from cholesterol and fat. The edible cultivars of banana are mainly propagated by vegetative means are of sterile triploid or tetraploid. The cultivated bananas are basically derived from inter- and intraspecific crosses with respect to diploid (2n=22) wild species viz. *Musa acuminate Colla* and *M. balbisiana Colla*. The cultivated bananas may be in diploid, triploids or tetraploids (Simmonds and Shepherd, 1955). The genome constitution of these is designated as AA (*M. acuminata*) or BB (*M. balbisiana*) in context of the chromosome sets.

Globally the genus *Musa* is of immense significance with reference to the commercial and nutritional value of the varieties cultivated. Phenotyping of various physiological characteristics that include biotic and abiotic stress tolerance, chiefly under monitored, restraint and conditions and reproducible conditions, is hard because of the size of the plants and their long-life cycle.

The genetic diversity of banana is extensively analyzed by DNA marker technologies for taxonomy, cultivar true-to-type assessment and the development of the genetic linkage map (Caliskan *et al.*, 2015). The most commonly used DNA markers are the internal transcribed spacer (ITS) of nuclear ribosomal DNA in plant phylogenetic and DNA barcoding analyses, the technique has been suggested as a core plant DNA barcode (Cheng

et al., 2016). ITS is widely used in plant molecular systematic studies at the generic and species levels as it has the potential to generate a high resolution of inter- and intraspecific relationships (Baldwin *et al*., 1995; Keller *et al*., 2010; Buchheim *et al*., 2011; Yuan *et al.,* 2015). In spite of the merits of ITS, it has a major drawback of universality and specificity of PCR primers for the ITS region which causes difficulty in amplification and sequencing (Cheng *et al*., 2016). Initially, ITS was proposed for barcoding flowering plants (Kress *et al*., 2005), but later the technique faded its popularity due to the incomplete concerted evolution of multiple copies, dissimilar traits from both parents, contamination of DNA from varied species (e.g. during symbiosis) and also technical issues. Later it was defined that these imperfections had no major impact and ITS was re-proposed as a barcode for seed plants (Hollingsworth 2011; Li *et al*., 2011; Song *et al*., 2012). Several methodological studies demonstrated that ITS is more efficient or to use a combination of barcodes from both the biparentally inherited nuclear genome and the uniparentally inherited plastid genome for reliable identification of species, and ITS is the most potential candidate from the nuclear genome (Chase and Fay 2009; Fazekas *et al.*, 2009; Roy *et al.*, 2010). The genetic diversity and evolutionary relationship within the *Musa* accessions can be determined by analyzing the internal transcribed spacer region (ITS1-5.8S-ITS2) of nuclear ribosomal DNA (Čížková *et al*., 2015). The family Musaceae has been phylogenetically reconstructed successfully employing ITS region (Li *et al.,* 2010; Liu *et al.,* 2010; Hřibová *et al.,* 2011). It is demonstrated that the genome verification for inter- and intraspecific banana hybrids can be verified by ITS locus (Hřibová *et al.,* 2011). The present investigation was aimed at evaluation of ITS 2 as the mini barcode in determining the phylogenetic relationship among the 11 varieties of *Musa* spp.

METHODS

Sample Collection

The 11 *Musa* cultivars used in this study were procured from different regions of Andhra Pradesh, representing *M. acuminate* and *M. acuminata* x *M. balbisiana.* All the collected plant materials were authenticated from the Department of Botany and Microbiology, Nagarjuna University, Guntur and respective voucher numbers (ANUH2001 to ANUH2011) were obtained for the same. Descriptive information of the plant material and the place of sample collection are listed out in Table 1.

Table 1: List of *Musa* Varieties Employed in the Study with their Ploidy

Sample No.	Local Name	Ploidy
S1	Red Banana	AAA
S2	Grand Naine Banana	AAA
S3	Robusta Banana	AAA
S4	Tella Chakkarakeli Banana	AAA
S5	Nanjangud Rasbale Banana	AAB
S6	Cooking Banana	ABB
S7	Nendran Banana	AAB
S8	Udhayam Banana	ABB
S9	KarpuraValli Banana	ABB
S10	Monthan Banana	ABB
S11	Elakki Banana	AB

DNA Extraction and Purification

Fresh young leaves were harvested for DNA isolation, washed with water and ethanol to remove external contaminants. The DNA extraction was performed employing Aristogene kit (Catalogue number: ARK-11). 400mg of leaves were cut into small pieces and homogenized with dry ice using tissue homogenizer. Finely ground tissue was transferred into 30 ml centrifuge tube and 15ml of lysis buffer was added. The tubes were incubated at 65˚C for 1 h 30 min in a water bath with intermittent mixing, followed by centrifugation at 10,000rpm for 10 min. The supernatant was carefully transferred into a fresh 30 ml centrifuge tube and an equal volume of chloroform was added and mixed well. The tubes were centrifuged at 10,000 rpm for 15 min. Without disturbing the interface, an aqueous layer was pipetted out into the fresh 30ml centrifuge tube. To the tube added equal volumes of isopropanol and $1/10^{th}$ volumes of 3M sodium acetate and mixed well. The tubes were incubated at room temperature for 5-10 min and later centrifuged at 10,000 rpm for 10-15 min. After discarding the supernatant, the pellet was washed with 1ml of 70% ethanol and air dried. Finally, the pellet was suspended in 400µl of 1X Tris- EDTA buffer. To remove inhibitors the DNA sample was further purified using column purification kit (Qiagen). The eluted DNA samples concentrations were determined by measuring A260nm using UV spectrophotometer (UV-1800 Shimadzu). The evaluation of purified DNA was done at A260/A280 ratio (Franco *et al.*, 2007). 2µl of DNA sample with 10µl double distilled water and 2µl of DNA loading dye was electro phoretically checked on 0.8% agarose gel.

ITS Region PCR Amplification and Identification

PCR amplification for ITS gene was performed with ITS 2 forward (ITS 2 FP 5'AGGAGAAGTCGTAACAAGGT'3) and reverse primer (ITS 2 RP 5'TCCTCCGCTTATTGATATGC'3). The PCR amplification of the ITS region was performed at a volume of 50µl of PCR mixture. The PCR amplification components were 21 µl double distilled water, 25 µl 2X PCR master mix, 1 µl primer and 2 µl of DNA template. All the reagents used were from Aristogene Biosciences Pvt. Ltd. The PCR conditions were initial denaturation at 94ºC for 2 minutes followed by denaturation at 94ºC for 30 seconds. Annealing at 55 ºC for 30 seconds and extension at 72ºC for 1 minute and a final extension for 72ºC for 5 minutes. This amplification was repeated for 30 cycles. The PCR products obtained was gel purified with Gel Elution Kit, ARK-07(Aristogene). The product was quantified and used for ITS region sequencing. The resultant sequences were subjected to BLAST search in the relevant database and the species were identified. The sequences were submitted to the National Center for Biotechnology Information (NCBI) database to obtain GenBank accession numbers.

Phylogenetic Analysis

The phylogenetic analysis was carried out based on rDNA ITS sequences employing MEGA version 6.06 software (Kimura, 1980; Tamura *et al.*, 2013). The sequences were aligned with ClustalW (1.6). The phylogenetic constructed employing Neighbor-Joining (NJ) method with the Maximum Composite Likelihood model and pairwise deletion for gaps/missing data treatment with 1000 replications for bootstrap.

RESULTS

The primer ITS 2 generated a minibarcode for all the 11 varieties of *Musa* spp. The ITSsequencing identification of 11 *Musa* varieties and their GenBank accession numbers obtained from NCBI is presented in Table 2. Figure 1 represents the phylogenetic tree constructed based on Neighbor-Joining method for the 11 *Musa* spp.

Table 2: ITS Sequencing Identification and Gen Bank Accession Numbers

Sample No.	ITS Sequencing identification	Gen Bank Accession Number	Base Pairs
S1	*Musa acuminata*	MG845588	611
S2	*Musa acuminata*	MG845589	619
S3	*Musa acuminata*	MG845590	510
S4	*Musa acuminata*	MG845591	426
S5	*Musa acuminata*	MG845592	427
S6	*Musa acuminata*	MG845593	516
S7	*Musa acuminata*	MG845594	595
S8	*Musa acuminata*	MG845595	645
S9	*Musa acuminata*	MG845596	548
S10	*Musa acuminata*	MG845597	636
S11	*Musa acuminata*	MG845598	609

ITS Sequences

>Seq1 [organism=*Musa acuminata*] Red Banana AAA group isolate ITS region, partial sequence

AACATTGTCGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCC
CGACAACACCCCCAATGTCGGTTCGCCCTCGGGCGGGACGATCGAGGGGATGAACTACCAACCCCG
GCGCGGATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCGCTGCATGCAGGCTACGATT
CCTACGGTGACCCCATTGGACGACTGTCGGCAACGGATATCTCGGCTCTCCCCTCGATGAAGAACGT
AGGGAAATGCGATACCTGGTGTGAATTGCAGAATCCCGTGAACCATCCAGTCTTTGAACGCAAGTTG
CGCCCGAGGCCATCCGGCTAAGGGGCCGCCTGCCTGGGCGGCCCGCTTTCGACGCTTGTTCGTTGCC
CCCTCGGGGGGGGGGTGGGGGCGAACGCGGAGGATGGCCCCCCCGTGCCGGAAGGTGCGGTTGGCC
GAAGAGCGGGCCGTCGGTGGTTGTCGAACACGACGCGTGGTGGATGCCTTGTGCGAGCCGTACGTC
GTGCCTTCGGGACCCGGGCGAGGCCTCGAGGACCCAAGTCGTGGTGCGAGTCGATGCCACGGACCG
CGACCCCCAGGAC

>Seq2 [organism=*Musa acuminata*] Grand Naine Bnanana AAA group isolate ITS region, partial sequence

GTCATTGTCGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCC
CGACAACACACCCCCCCGAATGTCGGTCCGCCCTCGGGCGGGACGACCGAGGGGATGAACTACCAA
CCCCGGCGCGGATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCGCTGCATGCAGGAG
GCTACAATTCCGACGGTGACCCCATTGGACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGAT
GAAGAACGTAGCGAAATGCGATACCTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGA
ACGCAAGTTGCGCCCGAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTT
CGTCGTTGCCCCCTCGGGGGGTGGGGGCGAACGTGGAGGATTGGGCCCCCCCTGCCGGAAAGGTGC
GGTTGGCCGAAGAGCGGGCCGTCGGTGGTTGTCGAACACGACGCGTGGTGGATGCCTTGTGCGAGC
CGTACGTCGTGCCTTCGGGACCCGGGCGAGGCCTCGAGGACCCAAGTCGTGGTGCGAGTCGATGCC
ACGGACCGCGACCCC-AGGTCAG

>Seq3 [organism=*Musa acuminata*] Robusta Banana AAA ITS region, partial sequence

CGAGACCCATTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGACAAC
ACCCCGATGTCGATCCGCCCTCGGGTGGGACGACGGAGGGGATGAACCACCAACCCCGGGGCGGAA

AGCGCCCAGGTACACGAACATCGGAGTCGGAGGGCCTCTCTGCATGCAGGAGGATACAATTCCGAC
GGTGACCCCATTGGATGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAAAACGTAGCGA
AATGCGATACCTGGTGTGAATTGCAAAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCC
GAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGCCGATGCCCCCTC
GGGGGGGTGGAGGCGTGTGCGGAGGATGGCCCCCCGTGCCCGAGGGTGCGGTTGGCCGAAAAGCG
GGCCGTCGGTGGTTGTCAAACACCACGCGTGGTGGATGCCTTGTGC

>Seq4 [organism=*Musa acuminata*] Tella Chakkarakeli Banana AAA group isolate ITS region, partial sequence

GCGGAGGACTTGTCGAGACCACTGACGAGGCGACCGTGATGCGTCAACGATTGCTCGTCGGGCTCG
TCCCGACAACACCCCGAATGTCGGTTCGCCCTCGGGCGGGACGATCGAGGGGATGAACTACCAACC
CCGGCGCGGATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCGCTGCATGCAGGCTACG
ATTCCGACGGTGACCCCATTGGACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAA
CGTAGCGAAATGCGATACCTGGTGTGAATTGCAGAATCCCGTGAACCCTCGAGTCTTTGAACGCAAG
TTGCGCCCGAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGTCGTT
GCCCCCTCGGGGGGGGGGGGGGGGGG

>Seq5 [organism=*Musa acuminata*] Nanjangud Rasabale Banana AAB group isolate ITS region, partial sequence

GTCGAGACCACTGACGAGGACGACCGTGATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGACAAC
ACCCCCGAATGTCGGTCCGCCCTCGGGGGGGACGACCGAGGGGATGAACTACCAACCCCGGCGCGG
ATAGCGCCAAGGAACACAAACATCGAAGTCGGAGGGCCTCTCTGCATGCAGGAGGCTACAATTCCG
ACGGTGACCCCATTGGACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGC
GAAATGCGATACCTGGGGTGAATTGAAAATCCCGTGAACCATCGAGTCTTTGAACGCAGGTTGCGC
CCGAGGCCATCCGGCAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGTGGT-
GCCCCCTCGGGGGGGGGGGGAACGTAAAATGTCTCTG

>Seq6 [organism=*Musa acuminata*] Cooking Banana ABB group isolate ITS region, partial sequence

CGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGACAAC
ACCCCGATGTCCATCCCCCCTCGGGTGGGACGACGGAGGGGATGAACTACCAACCCCGGGGCGGAA
AGCGCCCAGGTACACGAACATCGGAATCGGAGGGCCTCTCTGCATGCAGGAGGATACAATTCCGAC
GGTGACCCCATTGGATGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGA
AATGCGATACCTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCC
GAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCCACGCTTCGCCGATGCCCCCTC
GGGGGGGTGGAGGCGTGTGCGGAGGATGGCCCCCCGTGCCCGAGGGTGCGGTTGGCCGAAAAGCG
GGCCGTCGGTGGTTGTCAAACACGACGCGTGGTGGATGCCTTGTGCGAGCCG

>Seq7 [organism=*Musa acuminata*] Nendran Banana AAB group isolate ITS region, partial sequence

GTCGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGACA
ACACCCCGAATGTCGGTTTGCCCTCGGGCGGGACGATCGAGGGGATGAACTACCAACCCCGGCGCG
GATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCGCTGCATGCAGGAGGCTACAATTCC
GACGGTGACCCCATTGGACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTA
GCGAAATGCGATACCTAGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGC

GCCCGAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGTCGTTGCCC
CCTCGGGGGGGGGGCGAACACGGAGGATGGCCCCCCGTGCCGGAAGGTGCGGTTGGCGGAAGAGC
GGGCGGTCGGTGGTTGTCGAACACGACGCGTGGTGGATGCCTTGTGCGAGCCGTACGTCGTGCCTTC
GGGACCCGGGGGAGGCCTCGAGGACCCAAGTCGTGGTGCGAGTCGATGCCACGGACCGCGACCCC

>Seq8 [organism=Musa acuminata] Udhayam Banana ABB group isolate ITS region, partial sequence

TCGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGACAA
CACCCCCGAATGTCGGTCCGCCCTCGGGCGGGACGACCGAGGGGATGAACTACCAACCCCGGCGCG
GATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCGCTGCATGCAGGAGGCTACAATTCC
GACGGTGACCCCATTGGACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTA
GCGAAATGCGATACCTGGTGTGAATTGCAAAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGC
GCCCGAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGTCGTTGCCC
CCTCGGGGGGGGGGGGGCGAACGCGGAGGATGGCCCCCCGTGTCGGAAAGGTGCGGTTGGCCAAAG
AGCGGGCTGTCGGTGGTTCTCGAACACGACGCGGGGTGGATGCCTTGTGCGAGCCGTACGTCGTGCC
TTCGGAACCCGGGCGAGGCCTCGAGGACCCAAGTCGTGGTGCGAGTCGATGCCACGGACCGCGACC
CCAGGTCAGGTGGGGCTACCCGCTGAGTTTAAGCATATCAATAAGGCGG

>Seq9 [organism=*Musa acuminata*] Karpura Vali Banana ABB group isolate ITS region, partial sequence

GACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGGCAACACC
CCGAATGTCGGTCCGCCCTCGGGTGGGATGACCGAGGGGATGAACTACCAACCCCGGCGCGGATAG
CGCCAAGGAACACGAACATCAAAGTCGAAGGGCCTCGCTGCATGCAGGAGGCTACAATTCCGATGG
TGACCCCATTGGACAACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGTGAAA
TGCGATACCTAGTGTGAATTGCATAATCCTGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCTCGA
GGCCATCCGGCTAAGGGCATGCTTGCATGGGCGTCACGCTTTCGACGCTTCGCTGTTGCCCCCTCGG
GGTGGGGGCGAACACGGAGGATGGTCCCCTGTGCCGGAAAGTGCGGTTGGGCGAAAAGTGGGCCGT
CAGTGGTTGTCAAACATGACGCGCGGTGGATGCCTTGTGCGAGCAGCACGTCGTGCCTTCTGGACCT
GAGCGAGGCTTTGAGG

>Seq10 [organism=*Musa acuminata*] Monthan Banana ITS region, partial sequence

TCATTGTCGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCC
GACAACACCCCCGAATGTCGGTCCGCCCTCGGGCGGGACGACCGAGGGGATGAACTACCAACCCCG
GCGCGGATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCGCTGCATGCAGGAGGCTAC
AATTTCGACGGTGACCCCATTGGATGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGA
ACGTAGCGAAATGCGATACCTGGTGTGAATTGCAAAATCCCGTGAACCATCGAGTCTTTGAACGCA
AGTTGCGCCCGAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGTCG
TTGCCCCCTCGGGGGGTGGGGGCGAACGCGGAGGATGGCCCCCCGTGTCGGAAAGGTGCGGTTGGC
CAAAGAGCGGGCTGTCGGTGGTTCTCGAACACGACGCGTGGTGGATGCCTTGTGCGAGCCGTACGT
CGTGCCTTCGGAACCCGGGCGAGGCCTCGAGGACCCAAGTCGTGGTGCGAGTCGATGCCACGGACC
GCGACCCCAGGTCAGGTGGGGCTACCCGCTGAGTTTAAGC

>Seq11 [organism=*Musa acuminata*] Elakki Banana AB group isolate ITS region, partial sequence

GACCGTGAATGCGTCAACGATTGCTCGTCGGGCTCGTCCCGACAACACCCCCGAATGTCGGTCCGCC
CTCGGGCGGGACGACCGAGGGGATGAACTACCAACCCCGGCGCGGATAGCGCCAAGGAACACGAA
CATCGAAGTCGGAGGGCCTCGCTGCATGCAGGAGGCTACAATTCCGACGGTGACCCCATTGGACGA
CTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACCTGGTGTG
AATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCCATCCGGCTAAG
GGCACGCCTGCCTGGGCGTCACGCTTTCGACGCTTCGTCGTTGCCCCCTCGGGGGGTGGGGGCGAAC
GCGGAGGATGGCCCCCCGTGTCGGAAAGGTGCGGTTGGCCGAAGAGCGGGCTGTCGGTGGTTCTCG
AACACGACGCGTGGTGGATGCCTTGTGCGAGCCGTACGTCGTGCCTTCGGAACCCGGGCGAGGCCT
CGAGGACCCAAGTCGTGGTGCGAGTCGATGCCACGGACCGCGACCCCAGGTCAGGTGGGGCTACCC
GCTGAGTTTAAGC

DISCUSSIONS

The phylogenetic tree displayed the overlapping of *Musa* spp. genotypes into 3 clusters. In cluster, I the AAA, ABB, and AB were clustered together, in cluster II AAA and AAB are clustered while in cluster III ABBand AAA are grouped together. In the present study, the phylogenetic tree constructed by MEGA software employing the Neighbor-Joining (NJ) method could not differentiate the genotypes of 11 varieties of *Musa* species.

CONCLUSIONS

In the present study, the ITS sequencing could not differentiate the genotypes of 11 varieties of *Musa* spp.

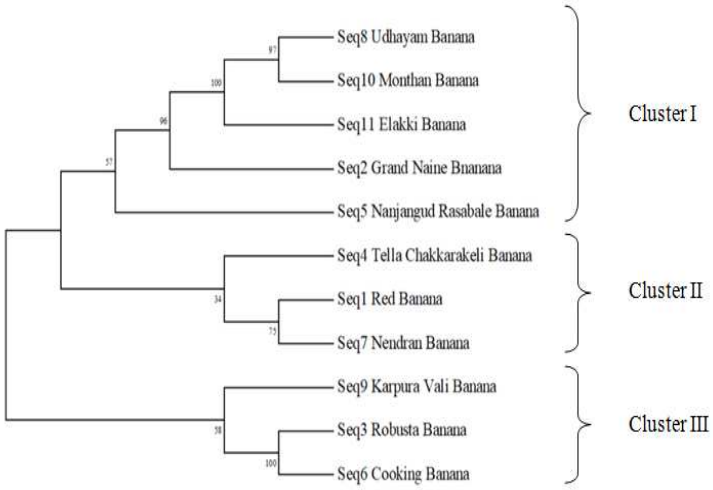

Figure 1: Phylogenetic Tree based on rDNA-ITS Sequences by Neighbour Joining Method

REFERENCES

1. *Baldwin BG, Sanderson MJ, Porter JM et al. (1995) The ITS region of nuclear ribosomal DNA - a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden, 82, 247–277.*
2. *Buchheim MA, Keller A, Koetschan C et al. (2011) Internal transcribed spacer 2 (nu ITS2 rRNA) sequence-structure phylogenetics: towards an automated reconstruction of the green algal tree of life. PLoS ONE, 6, e16931.*
3. *Chase MW, Fay MF (2009) Barcoding of plants and fungi. Science, 325, 682–683.*

4. *Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. Molecular ecology resources, 16(1), 138-149.*
5. *Čížková, J., Hřibová, E., Christelová, P., Van den Houwe, I., Häkkinen, M., Roux, N., ... & Doležel, J. (2015). Molecular and cytogenetic characterization of wild Musa species. Plos one, 10(8), e0134096.*
6. *FAO. FAO Production Yearbook, (2003). Food and Agriculture Organization of the United, New York, vol. 57, 2004. pp 340.*
7. *Fazekas AJ, Kesanakurti PR, Burgess KS et al. (2009) Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? Molecular Ecology Resources, 9, 130–13*
8. *Hollingsworth PM (2011) Refining the DNA barcode for land plants. Proceedings of the National Academy of Sciences of the United States of America, 108, 19451–19452.*
9. *Hřibová, E., Čížková, J., Christelová, P., Taudien, S., de Langhe, E., & Doležel, J. (2011). The ITS1-5.8 S-ITS2 sequence region in the Musaceae: structure, diversity and use in molecular phylogeny. PLoS One, 6(3), e17863.*
10. *Keller A, Wolf M, Dandekar T (2010) Ribosomal RNA phylogenetics: the third dimension. Biologia, 65, 388–391.*
11. *Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.*
12. *Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America, 102, 8369–8374.*
13. *Li D-Z, Gao L-M, Li H-T et al. (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. Proceedings of the National Academy of Sciences of the United States of America, 108, 19641–*
14. *Li, L. F., Häkkinen, M., Yuan, Y. M., Hao, G., & Ge, X. J. (2010). Molecular phylogeny and systematics of the banana family (Musaceae) inferred from multiple nuclear and chloroplast DNA fragments, with a special reference to the genus Musa. Molecular Phylogenetics and Evolution, 57(1), 1-10.*
15. *Liu, A. Z., Kress, W. J., & Li, D. Z. (2010). Phylogenetic analyses of the banana family (Musaceae) based on nuclear ribosomal (ITS) and chloroplast (trnL-F) evidence. Taxon, 59(1), 20-28.*
16. *Manzo-Sánchez, G., Buenrostro-Nava, M. T., Guzmán-González, S., Orozco-Santos, M., Youssef, M., & Medrano, R. M. E. G. (2015). Genetic Diversity in Bananas and Plantains (Musa spp.). In Molecular Approaches to Genetic Diversity. InTech.*
17. *Ahmed, S. J., Yas, N. K., & Hatem, H. A. Dna Polymorphism Of Interleukin Il-4 Of Nasal Mucosal Stem Cells In Nasal Polyps Of Iraqi Patients.*
18. *Roy S, Tyagi A, Shukla V et al. (2010) Universal plant DNA barcode loci may not work in complex groups: a case study with Indian Berberis Species. PLoS ONE, 5, e13674.*
19. *Simmonds, N, & Wheatherup, T. (1990). The taxonomy and origins of cultivated bananas (Musa). New Phytologist, 115, 567-571.*
20. *Song J, Shi L, Li D et al. (2012) Extensive pyrosequencing reveals frequent intra-genomic variations of internal transcribed spacer regions of nuclear ribosomal DNA. PLoS ONE, 7, e43971.*
21. *Swain, S., Das, S., Munsi, P. S., Lenka, P. C., Rout, G. R., & Swain, D. (2016). Molecular diversity study on dessert banana genotypes (Musa spp.) from Odisha using ISSR markers. International Journal of Agriculture, Environment and Biotechnology, 9(4), 513.*

22. *Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution, 30(12), 2725-2729.*

23. *Yuan QJ, Zhang B, Jiang D et al. (2015) Identification of species and materia medica within Angelica L. (Umbelliferae) based on phylogeny inferred from DNA barcodes. Molecular Ecology Resources, 15, 358– 371.*